The use of *Bacillus thuringiensis* on Forest Integrated Pest Management

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Abstract: *Bacillus thuringiensis* is a major microbial insecticide and a source of genes encoding several proteins toxic to insects. In this paper the authors give a brief summary of *Bacillus thuringiensis* used on the integrated pest management in forestry. The derivatives of Bt strain HD1 subsp *kurstaki* have been widely used to control the forest pests such as the gypsy moth (*Lymantria dispar*), spruce budworm (*Choristoneura fumiferana*), the pine processionary moth (*Thaumetopoea pityocampa*), the European pine shoot moth (*Rhyacionia buoliana*) and the nun moth (*Lymantria monacha*). Some progresses of transferring and expressing Bt toxin gene in forest trees are offered with a discussion on the limits and future prospects of using Bt products in forestry.

Key words: *Bacillus thuringiensis;* Integrated pest management; Forest protection
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Introduction

The concept of integrated pest management (IPM) was developed in 1950s and 1960s to address the agronomic problems caused by the use of broad-spectrum chemicals such as insecticide resistance, pest resurgence and secondary pest outbreaks. IPM is an ecological approach to pest control, which requires selective agents that are effective on target-pest species, yet preserves natural enemies and retains their contribution to overall control. In the context of plant protection, bio-pesticides are a key component of integrated pest management programs.

Ishiwata discovered a *Bacillus* spp attacking the silkworm in 1901. This is the first time of identifying the bacterial pathogens of insects in the world. Berliner discovered the same species infecting flour moths in Germany in 1915, and named the organism *Bacillus thuringiensis*, after the German province of Thueringen (Feitelson *et al.* 1996). *Bacillus thuringiensis* is a Gram-positive soil bacterium characterized by the ability to produce crystalline inclusions during sporulation. These inclusions consist of proteins that have insecticidal activity against several orders of insects. The insecticidal protein crystals are also called δ-endotoxins (Hofte and Whiteley 1989). It is thought that these toxins cause osmotic imbalance by creating pores in the cell membrane of the midgut epithelium of susceptible insects (McClintock *et al.* 1995)

In the 1950s, the first Bt products were commercialized in the United States for control of a variety of caterpillar pests attacking crops and forests (Bryant 1994). Great advances have been made with Bt products. Products based on the different strains of Bt are the most widely used in biological control agents in Europe, the United States and Canada (Masson *et al.* 1990; Evans *et al.* 1991). The revolution in molecular biology and genetic engineering allowed the unique and highly potent δ-endotoxins produced by Bt to be manipulated in a variety of ways that substantially enhance their utility and performance (Cannon 1993).

Application of Bt Products in Forestry

Most Bt-based bio-insecticide products are produced by using naturally occurring strains of Bt, and utilize only a small fraction of the known Cry proteins. In the United States and Canada, derivatives of Bt

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strain HD1 subsp kurstaki have become the major pesticide used to control the gypsy moth (*Lymantria dispar)* and the spruce budworm (*Choristonecera fumiferana*) respectively (Reardon1991). Other forest pests controlled by Bt include the nun moth (*Lymantria monacha*), Asian gypsy moth (*L. dispar*), pine processionary moth (*Thaumetopoea pityoeampa*), and the European pine shoot moth (*Rhyacionia buoliana*) (Bryant and Yendol 1988; Kleiner *et al.* 1995).

One of the most successful examples of Bt products to control forest pest happened in Poland, which is a major softwood producer. Nun moth is one of the most serious pests in Polish coniferous forests. The larvae are major defoliators, feeding particularly off young leaves. Infestations are usually cyclical with serious outbreaks occurring about every 50-60 years. Unexpectedly, though, in the spring of 1994 over 600 000 hm^2 of Polish forestry became seriously infested. This had not been predicted, since the last serious outbreak had only been 12 years earlier. It was postulated that the usage of broad-spectrum chemical insecticides to control the previous outbreak had made the forest more vulnerable to attack and also eliminated key parasites and predators of the nun moth, thus enabling the pest to increase in numbers more quickly, and effectively shortening the outbreak cycle.

A spray program was taken with a special forestry formulation of Btk called Foray 48B, from Novo Nordisk accounting for almost 25% of the total insecticide used in forest systems. This material is suitable for spraying from aircraft or helicopters at volumes as low as $4L/hm^2$ when applied through ultra-low volume (ULV) spray equipment. The results were excellent, with Btk providing about 95% control of the pest on average with minimal impact on beneficial and non-target organisms. Smaller, follow-up spray programs in 1995 and 1996 have effectively finished the job, and one would hope that the pest cycle in Poland has now been returned to its more natural 50-yr pattern (Carlton, 1992).

The nun moth causes similar problems in adjacent countries, in particular, Germany, Belarus, Ukraine, the Baltic Republics, and the Czech Republic. The Polish project has acted as a model for these other countries, and similar programs utilizing Btk are now in place, fore example, in Belarus (Li *et al.* 1995).

For some years now, products based on Btk have become the first choice for lepidopteran pest control in North American forestry, especially for gypsy moth (*Lymantria dispar*) outbreaks (Sundaram *et al.* 1996). This trend is quickly spreading to Europe, where Btk is increasingly perceived as a highly effective and environmentally benign forestry insecticide (Maczuga and Meirzejewski 1995).

Transgenic trees expressing toxins from Bt

The discovery of deoxyribose nucleic acid (DNA) as the basic molecule of the gene, and the deciphering of the genetic code, about the middle of the 20^{th} century, provided hope that plant breeders would be able to utilize genetic variation from many species in the improvement of crop plants. There have been 96 genes that code for δ-endotoxins described so far. The first insecticidal protein-encoding gene from Bt was cloned, sequenced, and expressed in *Escherichia coli* in 1981 (Schnept and Whiteley 1981). This provided the prospects for using these and other insecticidal proteins in transgenic plants. The first reported on use of the δ-endotoxin gene expressed in plants for insect control occurred in 1987. Tobacco plants, *Nicotiana tabacum* L., were developed that produced enough of the endotoxin to kill first-instar tobacco hornworm (*Manduca Sexta* L.) larvae placed on leaves of transformed plants (Andrews *et al.* 1987).

In forestry, the δ-endotoxin of Bt in the form of $CaMV^{35}S$-Bt was stably transformed by electric discharge particle acceleration into *Populus alba* $\times$ *Populus grandidentata* Crandon and *Populus nigra* Betulifolia $\times$ *Populus trichocarpa* hybrids (McCown *et al.* 1991). Transformed plants were highly resistant to feeding by the forest tent caterpillar (*Malacosoma disstria* Hubner) and the gypsy moth (*Lymantria dispar* L.). Hybrid *Populus* plants (clone NC 5339), genetically engineered with a *cry*1A(a) δ-endotoxin gene, showed field resistance to forest tent caterpillar and the gypsy moth, in the form of reduced feeding and weight gain; However, mortality of late third-instar larvae of gypsy moth did not differ when fed on transgenic and control foliage (Howe *et al.* 1994; Kleinor *et al.* 1995).

The gypsy moth is a major pest of many forest trees around the world. Poplar trees, *P. nigra* L., have been genetically engineered to resist this pest in China by transforming plants with *A. tumefaciens* strains carrying a truncated gene from Bt driven by a CaMV35S promoter. Three transgenic clones were selected for resistance to gypsy moth and *Apochemia cineraius*, reduced morphological changes, and promising silviculture traits. These are under large-scale field evaluation in six provinces in China (Chen Ying *et al.* 1995). Plants of poplar *P. alba* $\times$ *P. grandidentata* cv Crandon have been transformed to contain a truncated gene from Bt, plus the maize gene Ac. Transgenic plants expressing Ac and callus containing the Bt gene were recovered. Transgenic plants containing a modified Bt gene have been produced by transformation and regeneration of excised leaves of poplar hybrid (Zheng *et al.* 1995).

Populus deltoids plants were transformed using *A. tumefaciens* LBA 4404 strains containing a gene from Bt, and two of three plants regenerated successfully integrated the Bt gene.

The transfer and expression of the Bt toxin gene via *A. rhizogenes*-mediated transfer has been documented using Southern, Northern, and Western blots of needle tissue from transgenic plants of European larch trees (*Larix deciduas)* (Shin *et al.* 1994).

One of the key concerns involving transgenic plants expressing Bt toxins is the potential for resistance development in target insects (Tabashnik 1994; Tabashnik 1995). Continuous exposure to the toxin challenge has a high probability for selection of resistant individuals. Strategies have been developed to delay and ameliorate the onset of resistance involving establishment of refugia and high expression of Bt protein toxins in engineered plants (Van Rie *et al.* 1990; Roush 1997). The prospects for genetic engineering of insect resistance in forest trees was reviewed by Strauss et al. in 1991. They suggested that, in addition to the Bt genes, other potential strategies could include proteinase-inhibitor genes, chitinase, lectins, and baculovirus genes (Strauss *et al.* 1991; Metcalf, 1996).

Discussion

Bt products have been in commercial use for over 40 years. However its use has been largely restricted. Specific characteristics that limit the wider use of Bt include limited host-range specificity, inability to target cryptic feeding pests, slow action compared to chemical insecticides, and lack of residual activity. Some approaches were used to improve these characteristics. More attention is being given to the discovery of new Bt strains with activity on insects not previously found to be susceptible to Bt in recent years. Current research is devoted to improving the production, formulation and application technologies. Efforts are also being made to optimize the impact of the agents by integrating them with other novel plant protection strategies (Trumble *et al.*; 1997 Bryant and Yendol 1988). As progress in biotechnology and discovery of new Bt toxins continue, people can look forward to increased use of Bt products and biological toxins expressed in transgenic plants.

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